

FIG 1A. PRODUCTION OF GP88 BY TUMORIGENIC AND NON-TUMORIGENIC CELLS

Cells

PC 3A 1246 3T3

Cell Lysate

CM



FIG 1B. GP88 mRNA EXPRESSION

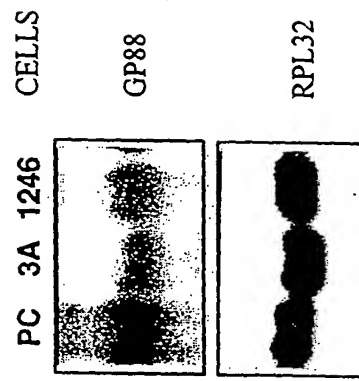
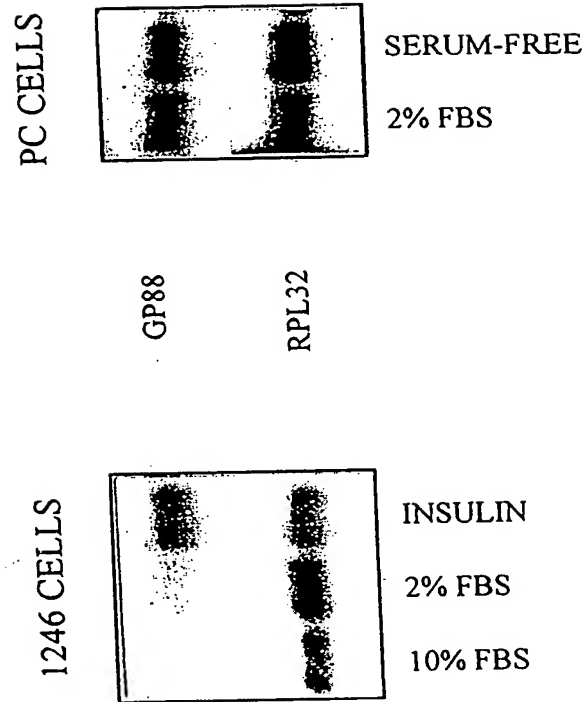


FIG 1C. GP88 mRNA EXPRESSION IN
VARIOUS CULTURE CONDITIONS



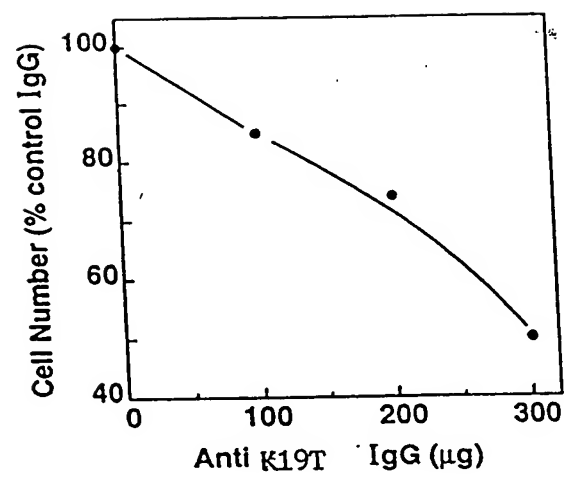
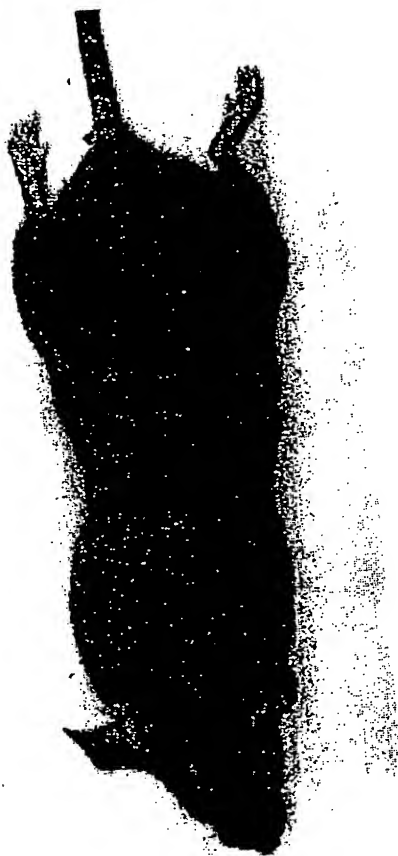


FIG. 2

FIG 3. ABSENCE OF TUMOR FORMATION IN C3H MICE BY INHIBITION OF GP88
EXPRESSION



GP88 ANTISENSE TRANSFECTED PC CELLS



CONTROL TRANSFECTED PC CELLS

FIG 4. GP88 PROTEIN EXPRESSION IN TUMOR
AND SURROUNDING TISSUES

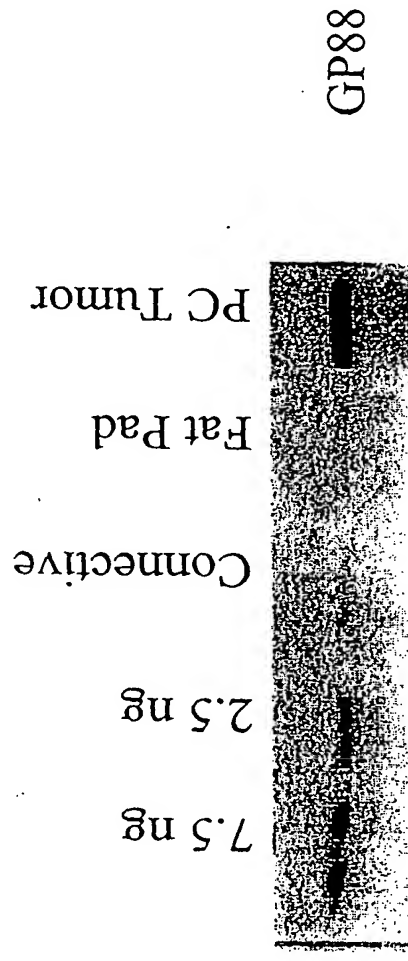
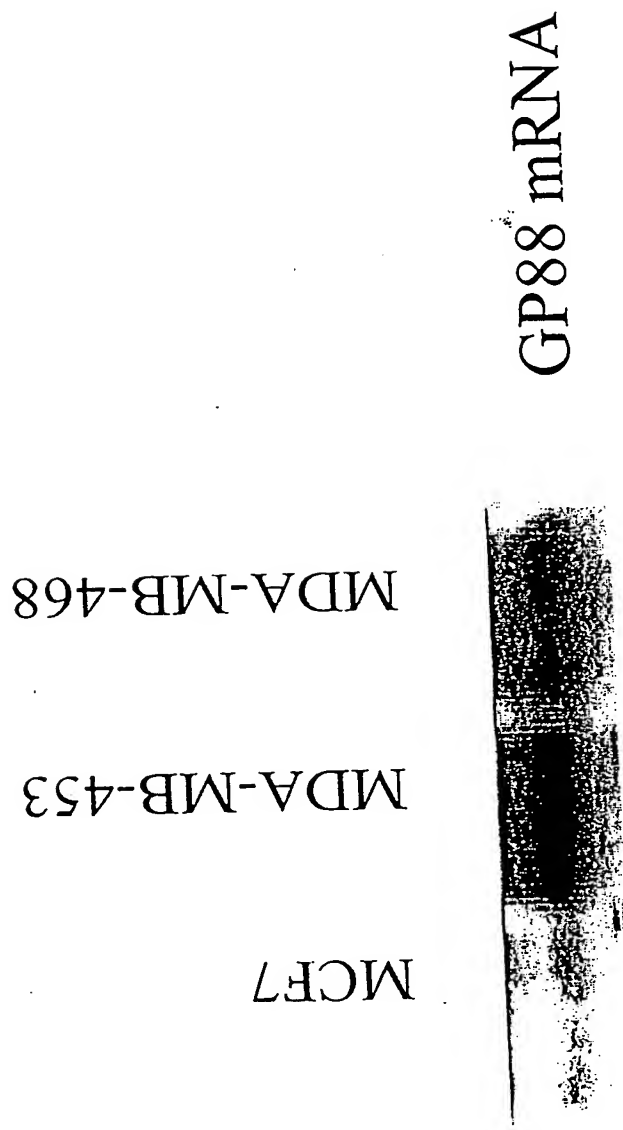


FIG 5. GP88 mRNA EXPRESSION IN
ESTROGEN-DEPENDENT AND INDEPENDENT
HUMAN MAMMARY CARCINOMA CELLS



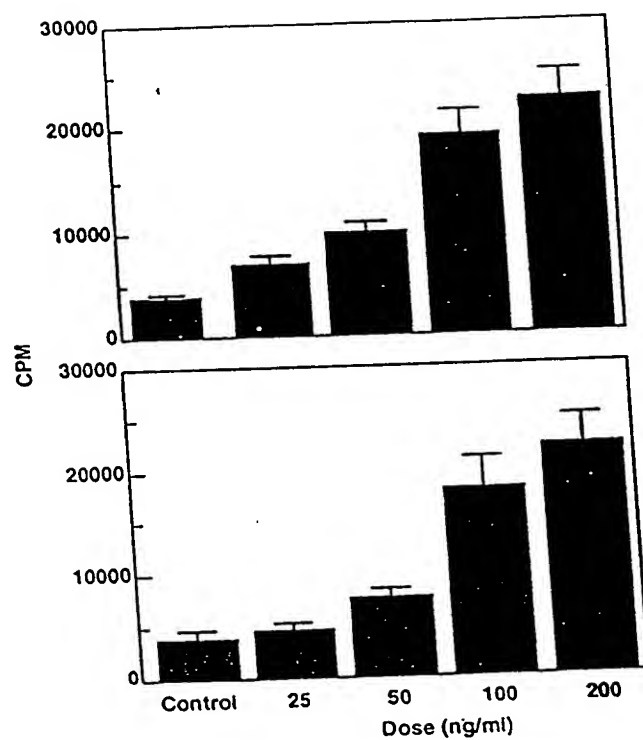
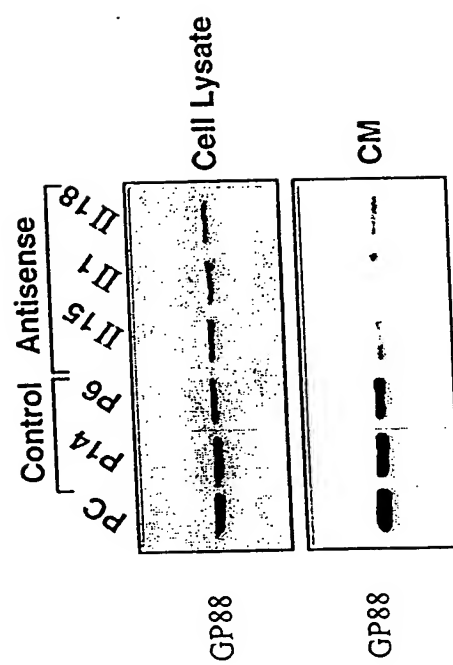


FIG. 6

FIG 7. EXPRESSION OF GP88 IN ANTISENSE AND
CONTROL TRANSFECTED PC CELLS



Mouse GP88 cDNA

[C	GGA	CCC	CGA	CCC	AGA	CAG	ACC	ATG	TGG	GTC	CTG	ATG	AGC	TGG	CTG	46
								M	N	V	L	M	S	W	L	8
GCC	TTC	GCG	GCA	GGG	CTG	GTA	GCC	GGA	ACA	CAG	TGT	CCA	GAT	GGG	CAG	94
A	F	A	A	G	L	V	A	G	T	Q	C	P	D	G	Q	24
TTC	TGC	CCT	GTT	GCC	TGC	TGC	CTT	GAC	CAG	GGA	GGA	GCC	AAC	TAC	AGC	142
F	C	P	V	A	C	C	L	D	Q	G	G	A	N	Y	S	40
TGC	TGT	AAC	CCT	CTT	CTG	GAC	ACA	TGG	CCT	AGA	ATA	ACG	AGC	CAT	CAT	190
C	C	N	P	L	L	D	T	W	P	R	I	T	S	H	H	56
CTA	GAT	GGC	TCC	TGC	CAG	ACC	CAT	GGC	CAC	TGT	CCT	GCT	GGC	TAT	TCT	238
L	D	G	S	C	Q	T	H	G	H	C	P	A	G	Y	S	72
TGT	CTT	CTC	ACT	GTG	TCT	GGG	ACT	TCC	AGC	TGC	TGC	CCG	TTC	TCT	AAG	286
C	L	L	T	V	S	G	T	S	S	C	C	P	F	S	N	88
GGT	GTG	TCT	TGT	GGT	GAT	GGC	TAC	CAC	TGC	TGC	CCC	CAG	GGC	TTC	CAC	334
G	V	S	C	G	D	G	Y	H	C	C	P	Q	G	F	H	104
TGT	AGT	GCA	GAT	GGG	AAA	TCC	TGC	TTC	CAG	ATG	TCA	GAT	AAC	CCC	TTC	382
C	S	A	D	G	N	S	C	F	Q	M	S	D	N	P	L	120
GGT	GCT	GTC	CAG	TGT	CCT	GGG	AGC	CAG	TTT	GAA	TGT	CCT	GAC	TCT	GCC	430
G	A	V	Q	C	P	G	S	Q	F	E	C	P	D	S	A	136
ACC	TGC	TGC	ATT	ATG	GTT	GAT	GGT	TGC	TGG	GGA	TGT	TGT	CCC	ATG	CCC	478
T	C	C	I	M	V	D	G	S	W	G	C	C	P	M	P	152
CAG	GCC	TCT	TGC	TGT	GAA	GAC	AGA	GTG	CAT	TGC	TGT	CCC	CAT	GGG	GCC	526
Q	A	S	C	C	E	D	R	V	H	C	C	P	H	G	A	168
TCC	TGT	GAC	CTG	GTT	CAC	ACA	CGA	TGC	GTT	TCA	CCC	ACG	GGC	ACC	CAC	574
S	C	D	L	V	H	T	R	C	V	S	P	T	G	T	H	184
ACC	CTA	CTA	AAG	AAG	TTC	CCT	GCA	CAA	AAG	ACC	AAC	AGG	GCA	GTG	TCT	622
T	L	L	K	K	F	P	A	Q	K	T	N	R	A	V	S	200
TTG	CCT	TTT	TCT	GTC	GTG	TGC	CCT	GAT	GCT	AAG	ACC	CAG	TGT	CCC	GAT	670
L	P	F	S	V	V	C	P	D	A	K	T	Q	C	P	D	216
GAT	TCT	ACC	TGC	TGT	GAG	CTA	CCC	ACT	GGG	AAG	TAT	GGC	TGC	TGT	CCA	718
D	S	T	C	C	E	L	P	T	G	K	Y	G	C	C	P	232
ATG	CCC	AAT	GCC	ATC	TGC	TGT	TCC	GAC	CAC	CTG	CAC	TGC	TGC	CCC	CAG	766
M	P	N	A	I	C	C	S	D	H	L	H	C	C	P	Q	248
GAC	ACT	GTA	TGT	GAC	CTG	ATC	CAG	AGT	AAG	TGC	CTA	TCC	AAG	AAC	TAC	814
D	T	V	C	D	L	I	Q	S	K	C	L	S	K	N	Y	264
ACC	ACG	GAT	CTC	CTG	ACC	AAG	CTG	CCT	GGA	TAC	CCA	GTG	AAG	GAG	GTG	862
T	T	D	L	L	T	K	L	P	G	Y	P	V	K	E	V	280
AAG	TGC	GAC	ATG	GAG	GTG	AGC	TGC	CCT	GAA	GGA	TAT	ACC	TGC	TGC	CGC	910
K	C	D	M	E	V	S	C	P	E	G	Y	T	C	C	R	296
CTC	AAC	ACT	GGG	GCC	TGG	GGC	TGC	TGT	CCA	TTT	GCC	AAG	GCC	GTG	TGT	958
L	N	T	G	A	W	G	C	C	P	F	A	K	A	V	C	312

FIG. 8

Mouse GP88 cDNA (continued)

TGT GAG GAT CAC ATT CAT TGC TGC CCG GCA GGG TTT CAG TGT CAC ACA	1006
C E D H I H C C P A G F Q C H T	328
GAG AAA GGA ACC TGC GAA ATG GGT ATC CTC CAA GTA CCC TGG ATG AAG	1054
E K G T C E M G I L Q Y P W M K	344
AAG GTC ATA GCC CCC CTC TGC CTG CCA GAC CCA CAG ATC TTG AAG AGT	1102
K V I A P L R L P D P Q I L K S	360
GAT ACA CCT TGT GAT GAC TTC ACT AGG TGT CCT ACA AAC AAT ACC TGC	1150
D T P C D D F T R C P T N N T C	376
TGC AAA CTC AAT TCT GGG GAC TGG GGC TGC TGT CCC ATC CCA GAG GCT	1198
C K L N S G D W G C C P I P E A	392
GTC TGC TGC TCA GAC AAC CAG CAT TGC TGC CCT CAG GGC TTC ACA TGT	1246
V C C S D N Q H C C P Q G F T C	408
CTG GCT CAG GGG TAC TGT CAG AAG GGA GAC ACA ATG GTG GCT GGC CTG	1294
L A Q G Y C Q K G D F M V A G L	424
GAG AAG ATA CCT GCC CCG CAG ACA ACC CCG CTC CAA ATT GGA GAT ATC	1342
E K I P A R Q T T P L Q I G D I	440
GGT TGT GAC CAG CAT ACC AGC TGC CCA GTA GGG CAA ACC TGC TGC CCA	1390
G C D Q H T S C P V G Q T C C P	456
AGC CTC AAG GGA AGT TGG GCC TGC TGC CAG CTG CCC CAT GCT GTG TGC	1438
S L K G S W A C C Q L P H A V C	472
TGT GAG GAC CCG CAG CAC TGT TGC CCG GCC GGG TAC ACC TGC AAC GTG	1486
C E D R Q H C C P A G Y T C H V	488
AAG GCG AGG ACC TGT GAG AAG GAT GTC GAT TTT ATC CAG CCT CCC GTG	1534
K A R T C E K D V D F I Q P P V	504
CTC CTG ACC CTC GGC CCT AAG GTT GGG AAT GTG GAG TGT GGA GAA GGG	1582
L L T L G P K V G N V E C G E G	520
CAT TTC TGC CAT GAT AAC CAG ACC TGT TGT AAA GAC AGT GCA GGA GTC	1630
H F C H D N Q T C C K D S A G V	536
TGG GCC TGC TGT CCC TAC CTA AAG GGT GTC TGC TGT AGA GAT GGA CGT	1678
W A C C P Y L K G V C C R D G R	552
CAC TGT TGC CCC GGT GGC TTC CAC TGT TCA GCC AGG GGA ACC AAG TGT	1726
H C C P G G F H C S A R G T K C	568
TTG CGA AAG AAG ATT CCT CGC TGG GAC ATG TTT TTG AGG GAT CCG GTC	1774
L R K K I P R W D M F L R D P V	584
CCA AGA CCG CTA CTG TAA GGA AGG GCT ACA GAC TTA AGG AAC TCC ACA	1822
P R P L L *	589
GTC CTG GGA ACC CTG TTC CGA GGG TAC CCA CTA CTC AGG CCT CCC TAG	1870
CGC CTC CTC CCG TAA CGT CTC CCC GGC CTA CTC ATC CTG AGT CAC CCT	1918
ATC ACC ATG GGA GGT GGA GCC TCA AAC TAA AAC CTT CTT TTA TGG AAA	1966
GAA GGC TGT GGC CAA AAG CCC CGT ATC AAA CTG CCA TTT CTT CCG GTT	2014
TCT GTG GAC CTT GTG GCC AGG TGC TCT TCC CGA GCC ACA GGT GTT CTG	2062
TGA GCT TGC TTG TGT GTG TGT GCG CGT GTG CGT GTG TTG CTC CAA TAA	2110
AGT TTG TAC GCT TTC TGA AAA AAA AAA	2137

A: Nucleotide sequence of human granulin/epithelin precursor (human GP88).
Human Granulin Genbank M75161\$

[cgaggcaga ccatgtggac cttgggtgagc tgggtggcct taacagcagg gctgggtgct
ggaacgagg gcccagatgg tcagttctgc cctgtggcct gctgcctgga cccggaggga
gccagctaca gctgctgccg tccccttctg gacaaatggc ccacaacact gagcaggcat
ctgggtggcc cctgccagg tcatgcccac tgcctgccc gccactcctg catctttacc
gtctcaggga cttccagttg ctgccccttc ccagaggccg tggcatgccc gcatggccat
cactgtctgc cagggggctt ccaactgcagt gcagacgggc gatcctgctt ccaaagatca
ggtaacaact ccgt]gggtgc catccagtgc cctgatatgc agttcgaat cccggacttc
tccacgtgct gtgttatggt ccatggctcc tgggggtgct gcccctatgcc ccaggcttcc
tgctgtgaag acagggtgca ctgctgtccg caggggtcct tctgcgacct ggttcacac
cgctgcatca caccacggg caccacccc ctggcaaaga agtccctgc ccagaggact
aacagggcag tggcctgtgc cagctcgggc atgtgtccgg acgcacggc ccgtgtccct
gatggttcta cctgctgtga gctgccagt gggaagatg gctgtgccc aatgcccac
gccacctgct gctccatga cctgcactgc tggcccaag acactgtgtg tgacctgatc
cagagtaagt gctctccaa ggagaacgt accacggacc tctcactaa gctgcctgcg
cacacagtg gcatgtgaa atgtgacatg gaggtgagct gccagatgg ctatccctgc
tgccgtctac agtgggggct ctgggggtgc tggccttta cccaggctgt gctgtgtgag
gaccacatac actgctgtcc cgcgggggtt acgtgtgaca cgcagaaggg tacctgtgaa
caggggcccc accaggtgcc ctggatggag aaggccccag ctcacctcag cctgccagac
ccacaagcct tgaagagaga tgtcccctgt gataatgtca gcagctgtcc ctcctccat
acctgtgcc aatcacgtc tggggagtgg ggctgtgtc caatcccaga ggctgtctgc
tgctcggacc accagcactg ctgccccag cgataacgt gtgtagctga ggggcaggt
cagcaggagaa gcagatcgt ggctggactg gagaagatgc ctgcccgcg cggttccta
tcccaccca gagacatcg ctgtgaccag cacaccagt gcccggtggg cggaacctgc
tgcccgagcc aggggtgggag ctgggcctgc tgcagttgc ccatgtgtg gctgtgcgag
gatcgccagc actgtgtccc ggctgtctac acctgcaacg tgaaggctcg atcctgcgag
aaggaaagtgg tctctgcca gcctgccacc ttcctggccc gtagccctca cgtgggtgtg
aaggacgtgg agtgtgggga aggacacttc tgcctgata accagacctg ctgcccagac
aaccgacagg gctgggctg ctgtccctac gccaggggcg tctgtgtgct tcatcggcg
cactgtgtc ctgtgtgctt ccgtgtgca cgcaggggta ccaagtgtt gcgcaggag
gccccgcgt gggacgccc ttgaggagc ccagcctga gacagctgt gtgagggaca
gtactgaaga cctgtgagcc ctgaggaccc cactcggagg gtgcctctg ctgaggcctc
cctagcacct cccctaacc aaattctccc tggacccat tctgagctcc ccatccat
gggaggtggg gcctcaatc aaggcccttc cctgtcagaa gggggtgag gcaaaagccc
attacaagct gccatccct ccccgttca gtggaccctg tggccagggt ctttcccta
tccacagggg tgttgtgtg ttgggtgtgc ttcaataaa gttgtcact tctt*

B: Amino-acid sequence of human granulin/epithelin precursor (human GP88).

MWTLVSWVALTAGLVAGTRCPDQGFCPVACCLDPGGASYSCCRP
LLDKWPTTLRHLGGPCQVDAHCSAGHSCIFTVSGTSSCCPFPEAVACGDGHHCCPRG
FHCSADGRSCFQRSGNNSVGAIQCPDSQFECPDFSTCCVMVDGSGWCCPMPQASCCED
RVHCCPHGAFCDLVHTRCITPTGTHPLAKKLPAQRTNRAVALSSVMCPDARSRCPDG
STCCELPSGKYGCCPMPNATCCSDHLHCCPQDTVCDLIQSKCLSKENATDILLTKLPA
HTVGDVVKCDMEVSCPDGYTCCRLQSGAWGCCPFTQAVCCEDHIHCCPAGFTCDTQKGT
CEQGP HQVPWMEKAPAHLSLPDPOALKRDVPCDNVSSCPSSDTCCQLTSGEWGCCPIP
EAVCCSDHQHCCPQRYTCVAEGQCQRGSEIVAGLEKMPARRGSLSHPRDIGCDQHTSC
PVGGTCCPSQGSWACCQLPHAVCCEDRQHCCPAGYTCNVKARSCEKEVVSQAQATFL
ARSPHVGVKDVCEGEGHFCHDNQTCCDRNRQGWACCPYAQGVCCADRRHCCPAGFRCA
RRGTKCLRREAPRWDAPLRDPALRQLL*

FIG 9

Mouse GP88 protein sequence

MSULMSLAFRAAGLVAG 17

TGCPDGGQF-CPVA-CCLDQG-GRNVSCNP LDTIPRITSHHL 57

0GSC-QTHGHCPAGV-SCLLTUSGTS-SCCPFSKGVSCGDGVHCCPQGFGHCSADGKSCFQNSDHP 120 9

GRUQCPGSGFECPSATCCIMVD-G-SHGSCPHQNSQCEDRVHCCPHGNSCDLVHTRCUSPTGIHTLLKKFPRAQKTHRAVSLPFS 204 1

UUCPRDKTQCPDOSTCCCLP-TGK-YGCCPHPHAI CCSDHLHCCPQDTUCDL IQSKCLSKNYTDLITKLPQYPUK 278 2,8

EUKC-DHEUSCPGVTCCALN-TGR-HGCCPFAKAVCCEDHHCQPAQGFDCHEKGTCEHGILQUPHMKU IAPRRLPDPQLKS 360 1,A

DIFCDQFIR-CPTHNJCCKLN-SGD-HGCCP IPEVCCSDHQHCCPQGF TCLAQGY-CQKSDTHVAGLEKIPARQITPLQIG 438 C

DIGCDQHI-SCEYGGDTCCPSLK-G-SHACCQLPHAVCCEDRQHCCPAGVTCNUKARTCEKUDVFIQPPULLGLPKUG 513 D

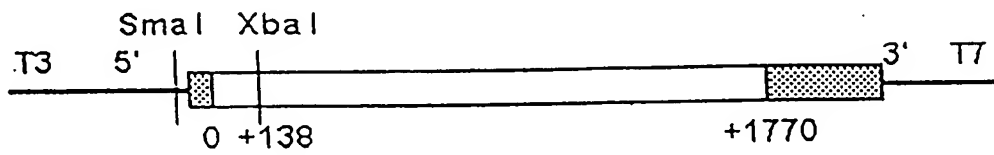
HUECGEGHF-CHDNQTCCKDSA-GV-HACCPYLKGVCCRDGRHCCPQGFHCSARGTCLRKKIPAUDHFLRDPUPAPLL 589 0

consensus sequence:

C.....C.....CC.....G.....CC.....CC..D..HCCP.....C.....C

1, 2: mouse epithelin 1, 2.
A, B, C, D, e, f, g: granulin A, B, C, D, E, F, G; N-terminus of granulin A, B, C, D have been sequenced.
Mouse epithelin precursor sequence is from Plowman et al.(1992).

hP88 cDNA Clone in Sk



Structure of pCMV₄ Expression Vector

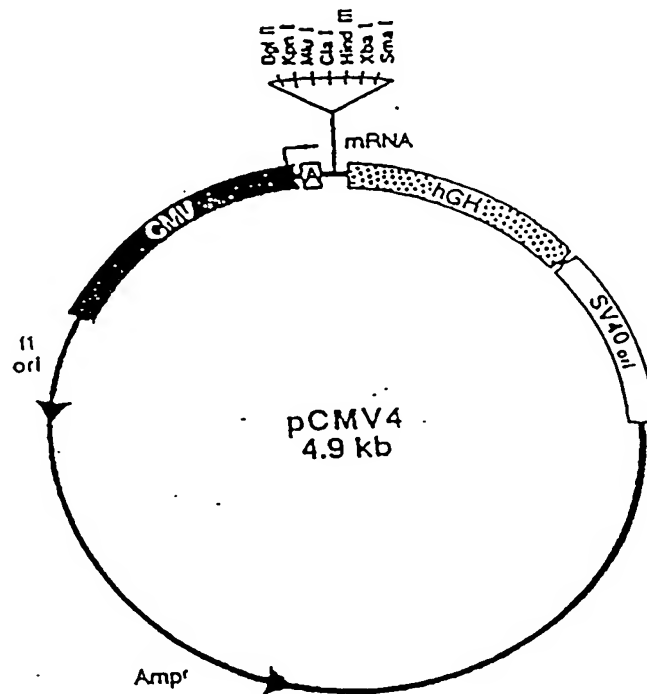


FIG. 11

FIG 12. CROSS-LINKING OF ^{125}I -rGP88 TO CCL64 CELLS

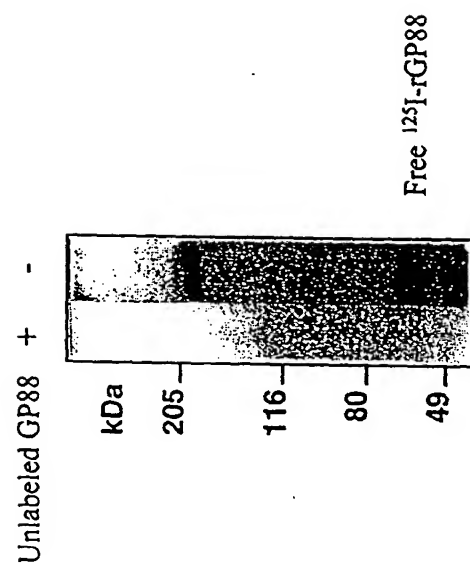
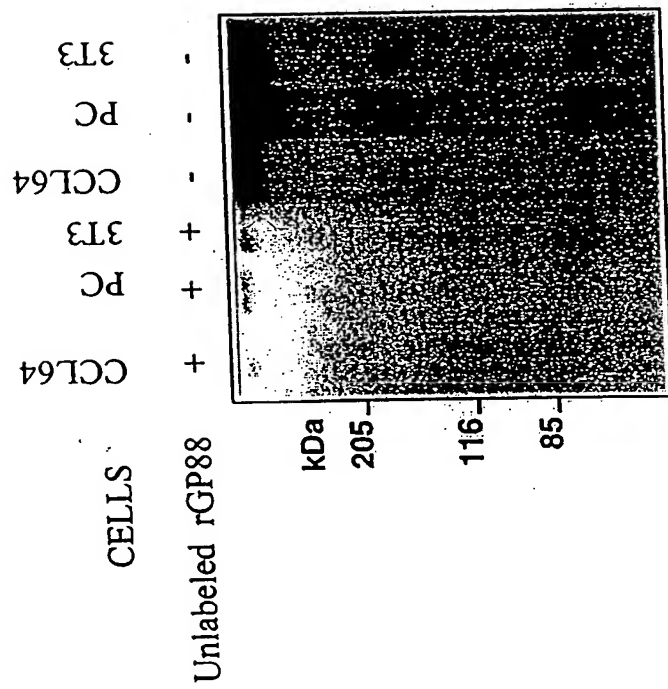
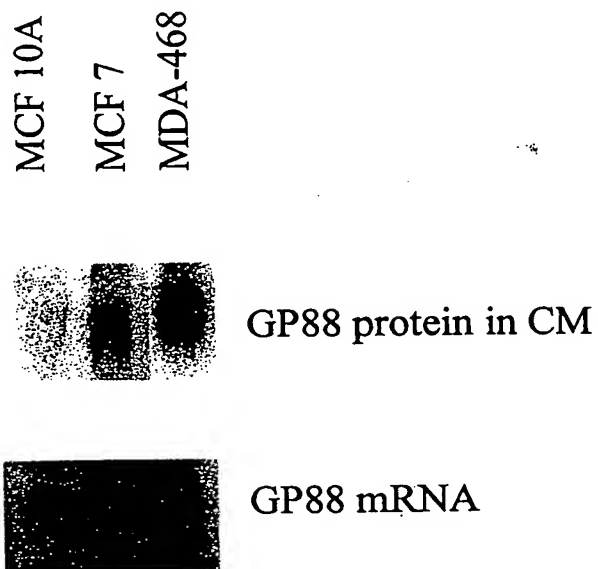


FIG 13. CROSS-LINKING OF ^{125}I -rGP88



Ad Fig 14 GP88 Expression In Non Tumorigenic (MCF 10A) And Malignant (MCF 7, MDA-468) Human Mammary Epithelial Cells



Ad Fig 15 GP88 Expression Is Inhibited By Antisense GP88 cDNA Transfection In Human Breast Carcinoma MDA-468

